# The Rat Probasin Gene Promoter Directs Hormonally and Developmentally Regulated Expression of a Heterologous Gene Specifically to the Prostate in Transgenic Mice

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**An expression cassette carrying 426 basepairs of the rat probasin (PB) gene promoter and 28 basepairs of 5′-untranslated region is sufficient to target the expression of the bacterial chloramphenicol acetyltransferase (CAT) gene specifically to the prostate in transgenic mice. The PB-CAT transgene was expressed in three of five (60%) independent lines of mice, and this expression, as reported previously for the endogenous rat gene, was male specific, restricted primarily to the lateral, dorsal, and ventral lobes of the prostate, with only very low levels of CAT activity detected in the anterior prostate and seminal vesicles. The developmental and hormonal regulation of the transgene also paralleled that reported for the rat gene, with a 70-fold increase in CAT activity in the mouse prostate observed between 2–7 weeks of age, a time corresponding to sexual maturation. PB-CAT activity in the prostate declined after castration to 3.5% of the precastration level, and the CAT activity in castrated males approached precastration levels when mice were supplemented with testosterone. Transgene expression in castrated males was not induced by dexamethasone. Coinjection of PB-CAT with a chicken lysozyme gene matrix attachment region resulted in their cointegration and further restricted the pattern of PB-CAT to the dorsolateral prostate, with suppressed expression observed in the ventral prostate. These studies demonstrate that a minimal rat probasin promoter can target heterologous gene expression specifically to the prostate in a developmentally and hormonally regulated fashion. (Molecular Endocrinology 8: 230–239, 1994)**

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## INTRODUCTION

Probasin (PB) is an androgen (1)- and zinc (2)-regulated protein first characterized in the dorsolateral prostate of the rat (3, 4). Immunohistochemistry with polyclonal and monoclonal antibodies has shown dual cellular localization of PB within the ducts and nucleus of epithelial cells of the prostate, and it has been demonstrated that both the nuclear and secreted forms of PB are translated from one bifunctional mRNA (3). Statistical analysis of the amino acid sequence predicted by sequencing the PB cDNA (3) has revealed that PB is related to rat $\alpha_2$-urinary globulin, rat odorant-binding protein, and bovine $\beta$-lactoglobulin, all members of a ligand carrier family of proteins. However, the ligand for PB has yet to be identified.

PB gene expression is developmentally regulated in

the prostate, and this expression is mediated in part by androgens. Expressed at low levels in the ventral prostate of the immature rat, PB mRNA levels decrease further in this tissue and increase significantly in the dorsolateral prostate with the onset of sexual maturity (2, 5). To characterize the molecular mechanisms regulating PB gene expression in the prostate, a 17.5-kilobase (kb) fragment carrying the structural rat PB gene with 0.5 kb of PB 5′-flanking DNA has been isolated (6). Using DNase-I footprinting analysis, two distinct androgen response elements (ARE-1 and ARE-2) have been identified in the PB 5′-flanking region, and glucocorticoid-responsive elements were also identified within the same DNA segment between nucleotides −235 and −286 relative to the PB transcriptional start site (6).

Transfection experiments have demonstrated that a −426/28 PB-chloramphenicol acetyltransferase (CAT) fusion gene is responsive to both androgens and glucocorticoids *in vitro*. To better define the sequences controlling the appropriate temporal and spatial expression of probasin *in vivo*, the PB-CAT gene was introduced into the germ line of transgenic mice. In this report, we demonstrate that the minimal −426/+28 PB promoter fragment carries sufficient information to direct developmentally and hormonally regulated expression of a heterologous gene specifically to the prostate in transgenic mice. Furthermore, this expression was both male specific and restricted to the epithelial cells of the lateral, dorsal, and ventral prostatic lobes. Because the level of transgene expression, but not the tissue distribution of expression, was highly dependent on the site of integration, studies were performed to determine whether a heterologous matrix attachment region (MAR) could buffer the PB-CAT transgene from the consequences of random transgene integration. The chicken lysozyme MAR was chosen for these studies because it has been previously demonstrated to mediate elevated and position-independent gene activity both *in vitro* (7, 8) and *in vivo* (9, 10). Cointegration of the MAR with PB-CAT resulted in prostate-specific CAT expression in all three (100%) founder lines, and this expression was further restricted to the dorsolateral prostate.

## RESULTS

### Generation of Transgenic Mice with the −426/28 Rat PB-CAT Fusion Transgene

To investigate whether the PB promoter fragment could impart cognate hormonal and developmental regulation in a spatially and temporally restricted manner to a heterologous gene, lines of transgenic mice were generated by microinjection of a 2.1-kb transgene carrying the −426/28 PB 5′-flanking sequences fused to the CAT reporter gene (Fig. 1). We chose the −426/28 PB fragment for our initial investigations because it had been shown in cell transfection experiments to respond

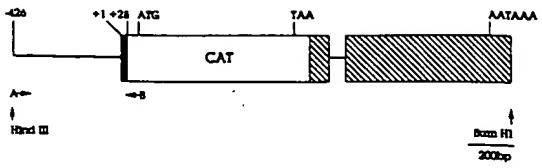


**Fig. 1.** Structure of the PB-CAT Fusion Gene Construct Used to Generate Transgenic Mice

The elements are: −426 to 1, the 5′-flanking region of the rat PB gene; 1–28, portion of the noncoding first exon of PB; ATG and TAA, the CAT open reading frame; *hatched region*, simian virus-40 sequences; and AATAAA, polyadenylation signals. The location and orientation of the primers used for PCR are denoted A and B (see *Materials and Methods*).

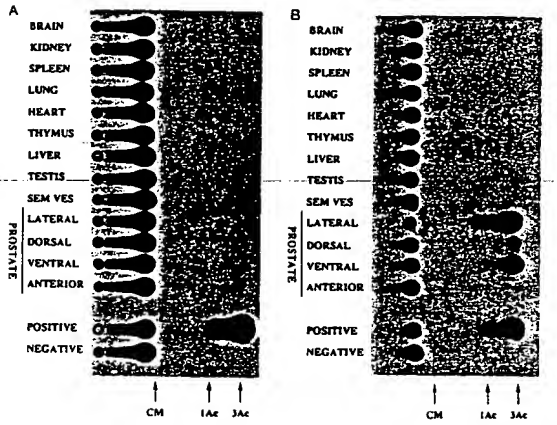


**Fig. 2.** Tissue-Specific Expression of PB-CAT in Transgenic Mice

CAT activity was measured in 25 μg protein obtained from the various tissues extracts of 7-week-old male mice of lines 4217(A) and 4248(B).

preferentially to androgens via two distinct androgen response elements (ARE-1 and ARE-2) (6). Five founder animals were identified by polymerase chain reaction (PCR) to carry the PB-CAT transgene; four of the founder mice were male, one was female, and all transmitted the transgene to their offspring in a Mendelian fashion.

### The PB-CAT Transgene Is Expressed in Prostate

To determine whether PB-CAT transgene expression was restricted to the prostate, male founder mice or male offspring of the female founder were killed at 7 weeks of age, and extracts were prepared from various tissues, as described in *Materials and Methods*. As shown in Fig. 2, PB-CAT expression in lines 4217 and 4248 was detected only in extracts prepared from prostate tissue. A similar pattern of expression, at a markedly reduced level, was observed in a third line, no. 4234 (data not shown). There was no CAT activity detected in extracts prepared from tissues obtained at necropsy of either virgin or lactating female mice (data not shown).

As shown in Table 1, the distribution and relative level of PB-CAT expression in line 4248 closely resemble those observed previously for endogenous rat PB mRNA (2). The PB-CAT transgene was expressed in a tissue-specific fashion in three of five lines examined, and CAT activities for these lines fell within a 2-log range, with line 4234 (25 copies) being the lowest, line 4217 (2 copies) showing intermediate expression, and line 4248 (one copy) showing the highest expression. Although the expression of the transgene did not appear to be copy number dependent, presumably due to position effects as a consequence of the site of transgene integration, it is important to note that all expressing lines exhibited consistent tissue-specific patterns of expression. Subsequent analysis has shown that the characteristic pattern of prostate-specific expression in line 4248 is maintained for at least 23 weeks (see below).

### PB-CAT Expression Is Restricted to Prostate Epithelium

Immunocytochemistry was employed to investigate the localization of CAT within the prostate (Fig. 3). As shown in Fig. 3B, immunohistochemical analysis revealed an intense pattern of staining that was concentrated toward the luminal border and confined within the cytoplasm of all prostatic epithelial cells. The expression of PB-CAT did not appear to be detrimental to development of the prostate, as the epithelium exhibited typical columnar morphology, the glandular luminae were wide and regular, and the interacinar tissue was loose and sparse. No positive staining was detected in either the epithelium or stroma in the samples obtained from the prostate of an age-matched control mouse (Fig. 3C). The antibody also detected weak PB-CAT expression in the epithelial cells of the ventral prostate that immediately border the urethra (Fig. 3D). Presumably, the concentration of CAT along the apical surface of the luminal prostatic epithelial cells is a consequence of the secretory function of these cells and the fact that the CAT gene does not encode a signal peptide that would target secretion of the protein.

**Table 1.** Relative Concentration of PB mRNA and PB-CAT Activities in Various Tissues

| Tissue | PB mRNA[a] | CAT[b] |
|---|---|---|
| Lateral Prostate | 100 | 100 |
| Dorsal Prostate | 33 | 6 |
| Coagulating Gland | 14 | 1 |
| Ventral Prostate | 4 | 22 |
| Seminal Vesicle | 2 | 3 |
| Testis | ND | ND |
| Brain | ND | ND |
| Heart | ND | ND |
| Liver | ND | ND |

ND, Not detected.
[a] Data refer to the endogenous rat PB gene (2).
[b] Data from triplicate determinations.

It has been demonstrated previously that both nuclear and secreted forms of PB can be translated from one bifunctional mRNA through the utilization of different translational start codons (3). Because the PB-CAT construct only carries the first 28 basepairs (bp) of the 5′-untranslated (5′UT) sequence, and the CAT gene does not encode a signal peptide, it was anticipated that translation would initiate at the AUG codon of the CAT gene, resulting in the cytoplasmic localization of the CAT gene product.

To further characterize the pattern of PB-CAT expression within the prostate and confirm the immunohistochemical observations that expression was preferential in the ductal epithelial cells, sections of prostate were analyzed by *in situ* hybridization (Fig. 4). The antisense CAT riboprobe detected CAT mRNA that was localized and evenly distributed within the cytoplasm of the epithelial cells of the dorsolateral and ventral lobes of the prostate (Fig. 4B). This pattern of epithelial cell expression is consistent with that observed for the endogenous PB mRNA in the rat gland (11). No hybridization was detected within the nuclei of the epithelial cells or in the stromal tissue, confirming the observations obtained by immunohistochemistry. Taken together, these observations illustrate that transcription of the PB-CAT construct and translation of PB-CAT mRNA are restricted to the epithelial cells of the prostate, and that the transgene displays a high degree of penetrance in transgenic mice, as demonstrated by the uniform pattern of expression throughout the ductal epithelium in the dorsolateral and ventral lobes.

### Developmental and Hormonal Regulation of the PB-CAT Transgene

The expression of the rat PB gene has been shown to be a marker for prostate growth and differentiation in the developing animal (2). In the rat dorsolateral prostate, the level of PB mRNA increases dramatically between 3–6 weeks of age (2), whereas in the rat ventral prostate, PB mRNA is detected at very low levels in the prepubertal gland and further decreases with sexual maturation (2). To determine the pattern of PB-CAT expression during development, extracts were prepared from the prostates of male mice between 2–23 weeks of age. As shown in Fig. 5, the level of PB-CAT expression increased 70-fold between 2–7 weeks, a time corresponding to the onset of sexual maturity. It is interesting to note that expression of PB-CAT in the ventral prostate of these mice increased approximately 2-fold between 10–23 weeks of age, whereas previous observations had shown PB mRNA levels to decrease in the ventral prostate of the postpubetal rat (2).

The rat PB gene is regulated by androgens (1, 2, 5), and it has been previously demonstrated that the androgen receptor is more active than the glucocorticoid receptor in activating PB-CAT expression *in vitro* (6). To determine whether the PB-CAT construct is regulated preferentially by androgens *in vivo*, sexually mature male transgenic mice were castrated, and levels of

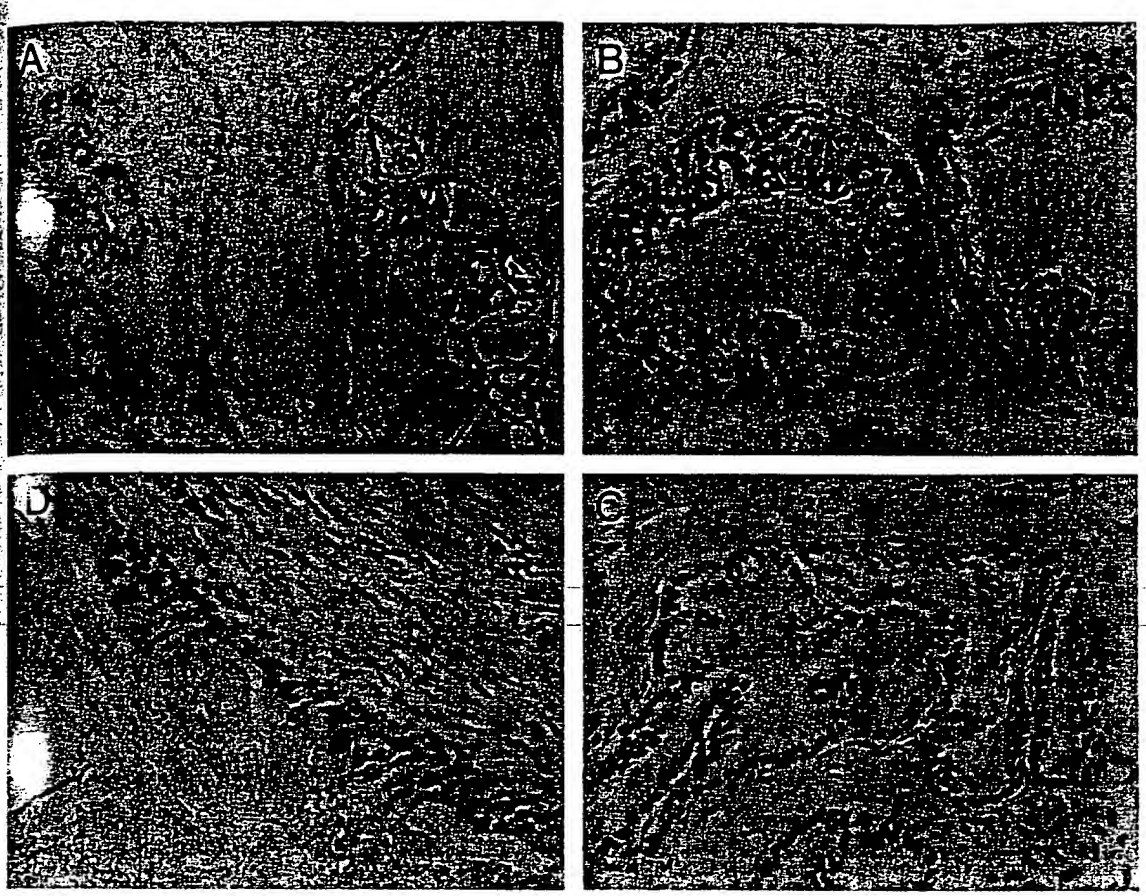


**Fig. 3.** Immunohistochemical Staining of PB-CAT Transgenic Mice

A, A survey photomicrograph of the bladder (*top*), urethra (*center*), and ventral (*right*) and lateral (*left*) prostate of a control mouse (magnification, ×40). B, Prominent cytoplasmic anti-CAT immunostaining is observed along the apical surface of ductal epithelial cells in the ventral prostate of transgenic mouse (magnification, ×400). C, Control showing no staining in prostatic epithelium of nontransgenic littermate (magnification, ×400). D, Diffuse staining along the prostatic-urethral boarder (magnification, ×400).

CAT activity were determined after either androgen or glucocorticoid replacement. As shown in Fig. 6, CAT activity decreased dramatically in the dorsolateral and ventral lobes after castration and returned proportionally to precastration levels after the administration of testosterone. Note that dexamethasone did not induce PB-CAT expression in these castrated mice.

In the 12-day castrated rat, it has been previously observed that levels of PB mRNA will return to precastration levels without a concomitant increase in prostate weight in what has been termed the postcastration rebound effect (11). Although the PB-rebound effect has not been characterized for the endogenous gene in the mouse, the level of PB-CAT transgene expression did not appear to significantly change, relative to that the control mice, in the 10-day castrated group (Fig. 6). The levels of PB-CAT activity did not increase with time, as evident from the levels observed in the 22-day castrated animals.

### A Heterologous MAR Influences the Pattern of PB-CAT Expression in Prostate

Although the prostate-specific pattern of expression was consistent, the observation that the level of PB-CAT transgene expression varied over 2-logs among the three independently founded lines carrying PB-CAT suggested that the transgene was sensitive to the site of integration and was probably missing *cis*-acting elements responsible for high level, copy number-dependent, site of integration-independent expression. In addition, the PB-CAT transgene was highly expressed in the ventral prostate of sexually mature transgenic mice, in contrast to the endogenous gene in the rat, further indicating that the transgene was missing an element(s) necessary for regulating the developmental switch that abrogates PB expression in the postpubetal ventral prostate. To determine whether the organization of chromatin at the transgene locus could be manipulated to influence the level of expression and appropriate

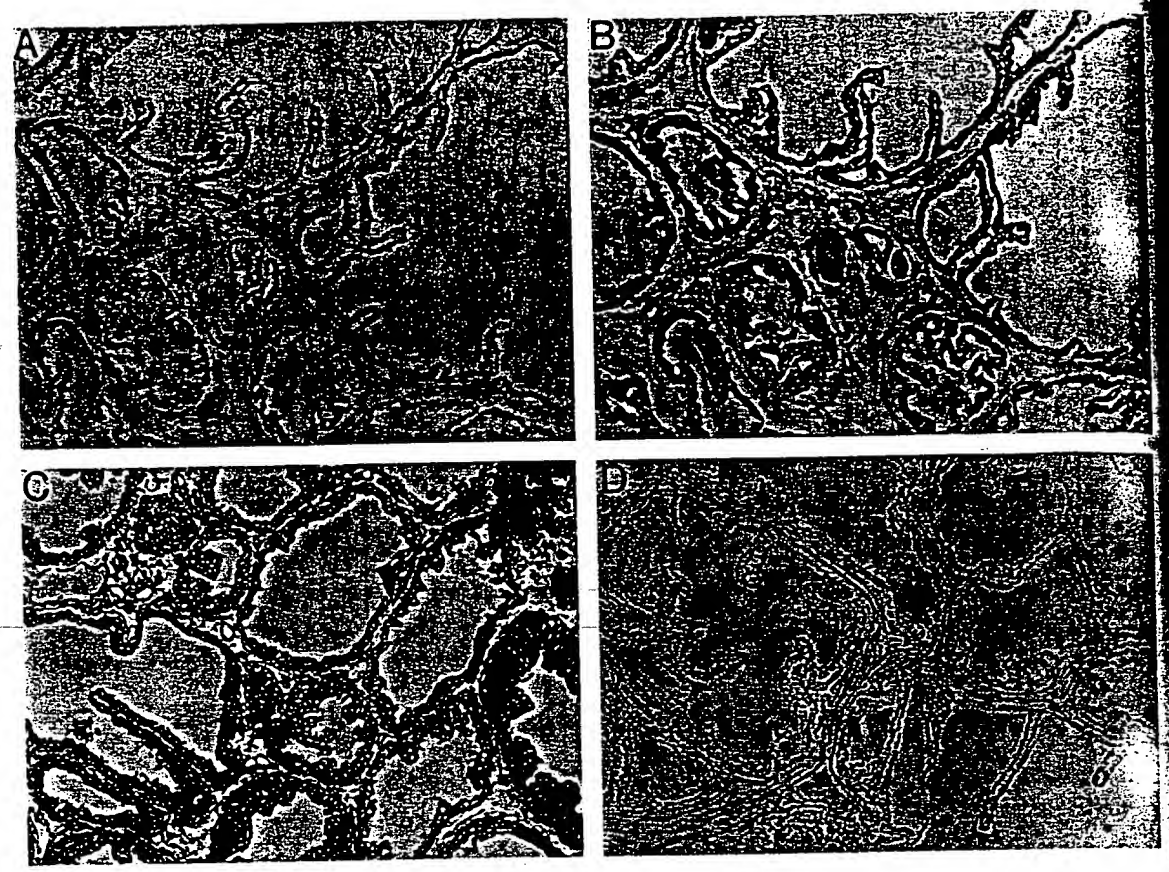


**Fig. 4.** *In Situ* Hybridization Histochemistry

Digoxygenin-labeled transcripts were hybridized to frozen serial sections and detected by alkaline phosphatase-conjugated antidigoxigenin antibody. A, Section through lateral prostate stained with hematoxylin and eosin (magnification, ×400). B, Detection of PB-CAT transcripts with an antisense CAT riboprobe (magnification, ×400). C, Detection of PB transcripts with an antisense PB riboprobe (magnification, ×400). D, The control shows no hybridization signal with a sense CAT riboprobe (magnification, ×400).

developmental regulation, the PB-CAT transgene was coinjected with a heterologous MAR. Derived from the up-stream flanking region of the chicken lysozyme gene; the MAR employed in these studies had been previously shown to mediate elevated and position-independent gene activity both *in vitro* (7, 8) and *in vivo* (9, 10). Analysis of founder mice or their progeny revealed that cointegration of the MAR with PB-CAT resulted in prostate-specific CAT expression in all three (100%) founder lines, and importantly, this expression was consistently restricted to the dorsolateral prostate and suppressed in the ventral prostate (Fig. 7). One line of mice (no. 5638) demonstrated an approximately 40% higher level of expression in lateral prostate compared to line 4248. However, a 10-fold variation in CAT expression was still observed between line 5638 and the other two lines of mice carrying PB-CAT and MAR despite the fact that all lines carried approximately the same number of MAR elements (data not shown). From these observations, it appears that random PB-CAT/MAR cointegration was not sufficient to facilitate high level and copy number-dependent expression. However, the MAR element was able to mediate integration-independent PB-CAT expression in all lines examined and further restricted expression to the dorsolateral prostate of sexually mature males.

## DISCUSSION

Together with the seminal vesicles, genital ducts, and bulbourethral glands, the prostate is a component of the accessory reproductive glands of the male reproductive system. In the rat and mouse, the prostate is composed of four anatomically distinct regions, namely the ventral, dorsal, lateral, and anterior (coagulating gland) lobes, with the dorsal and lateral lobes often examined together as the dorsolateral prostate (5). The prostate is dependent primarily on androgens for growth and differentiation during development and sexual maturation (12, 13).

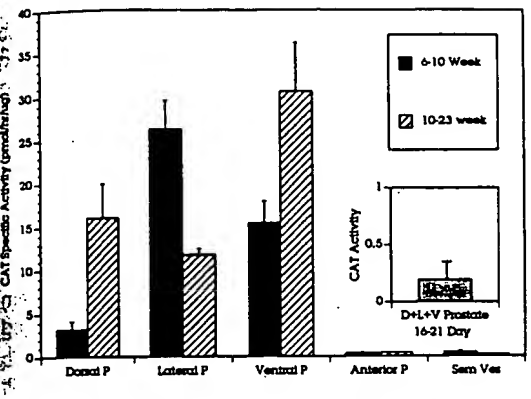


**Fig. 5.** Developmental Expression of PB-CAT in Transgenic Mice

CAT activities were determined from the dorsal, lateral, ventral, and anterior prostate and the seminal vesicles of 6- to 10-week-old (■) and 10- to 23-week-old (▨) transgenic mice of line 4248. *Inset*, CAT activity in dorsal and lateral and ventral prostate in 2- to 3-week-old transgenic mice of line 4248. *Error bars* show ±SEM.

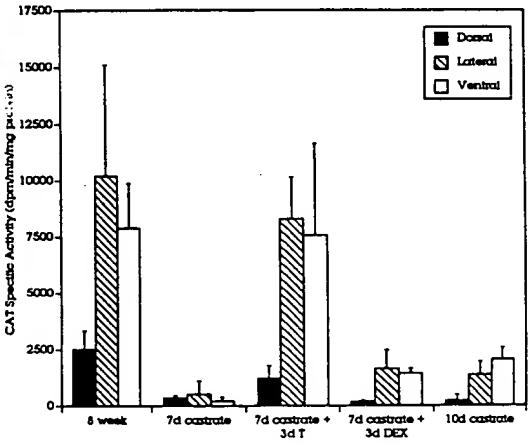


**Fig. 6.** The PB-CAT Transgene Responds to Androgens

CAT activities were determined in the dorsal (■), lateral (▨), and ventral (□) prostates of transgenic mice of line 4248 at the times indicated. Mice castrated at 8 weeks of age were supplemented with testosterone (T), dexamethasone (DEX), or vehicle, as described in *Materials and Methods*. *Error bars* show ±SEM.

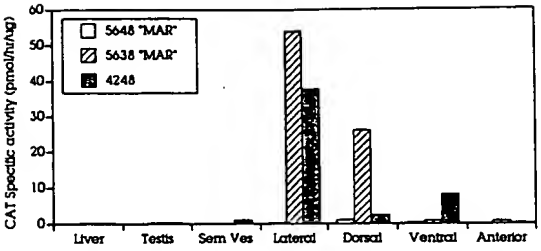


**Fig. 7.** Heterologous MAR Elements Influence Spatial Expression of PB-CAT in Transgenic Mice

CAT activities in various tissues were determined in 8-week-old transgenic mice of lines 5648 (PB-CAT+MAR; □), 5638 (PB-CAT+MAR; ▨), and 4248 (PB-CAT; ■).

As the dorsolateral lobes of the murine prostate are considered the most homologous to the peripheral zone of the human prostate (14), where approximately 68% of human prostate cancers are thought to originate (15), we have focused our attention on the molecular mechanisms regulating expression of the PB gene. Although tissue culture techniques are amenable to the identification of *cis*-acting sequences responsible for hormonal regulation of the PB gene (6), the introduction of a PB-CAT fusion gene into the germ line of mice permitted characterization of the molecular mechanisms governing the hormonal and developmental regulation of PB gene expression in the differentiating prostate. We chose the −426/28 PB fragment for our investigations because it had been shown in previous cell transfection experiments to respond preferentially to androgens, and two hormone receptor-binding sites were localized within this fragment (6). Even though a −286/28 PB fragment had demonstrated approximately 5-fold higher androgen-induced CAT activity in PC-3 cells cotransfected with androgen receptor (6), we believed that the longer −426/28 PB fragment would have a higher probability of carrying the necessary *cis*-acting elements to direct prostate-specific gene expression in transgenic mice.

The findings reported here demonstrate that the −426/28 PB fragment was indeed sufficient to direct prostate-specific expression of a heterologous gene and is the shortest fragment that has demonstrated ability to target expression of heterologous gene specifically to the prostate in transgenic mice and maintain the spatial pattern of transgene expression independent of the random nature of transgene integration. When a 9.5-kb fragment carrying the rat C3(1) gene with 4 kb of up-stream and 2 kb of down-stream flanking sequence was introduced into transgenic mice, the tissue distribution of C3(1) expression appeared to be sensitive to position effects, because expression was also observed in the testis of one line of mice and in the heart, skeletal muscle, and lung of female offspring of another (16). When a fragment of the C3(1) gene carrying 6 kb of 5′-flanking and only 80 bp of 5′UT sequence was fused to a heterologous β-galactosidase (βgal) reporter gene, the spatial pattern of C3(1)-βgal fusion transgene expression was not uniform (17). It is interesting to note that the −6 kb/80 C3(1) fragment appeared to be as tissue specific as the 9.5 kb C3(1) transgene, despite the fact the C3(1)-βgal fusion transgene did not contain a 0.35-kb 3′-region of the first C3(1) intron previously demonstrated to bind androgen receptor and impart androgen sensitivity to a heterologous promoter *in vitro* (18).

The developmental and hormonal regulation of the PB-CAT transgene closely parallels that of the endogenous rat PB gene. In the 3-week-old rat, dorsolateral PB mRNA accounts for almost 50% of the level found

in the sexually mature 6-week-old animal (2). On the other hand, in transgenic mice, a 70-fold increase in dorsolateral PB-CAT expression was observed between 2–7 weeks of age, a time corresponding to sexual maturation. Although the mouse PB gene has not been characterized, and differences may exist between the pattern of mouse and rat PB gene expression, we presume that additional 5′-flanking sequences may be necessary to direct expression in a temporally and spatially restricted manner at levels observed previously in the immature rat. To demonstrate that the increase in PB-CAT expression observed in transgenic mice between 2–7 weeks of age was related to the levels of circulating androgen, sexually mature male mice were evaluated for effects of hormonal manipulation by castration, followed by androgen or glucocorticoid replacement. The demonstration that CAT activity approached precastration levels when mice were supplemented with testosterone indicates that the PB-CAT transgene was responding to androgens *in vivo*. Although the −426/28 PB fragment responds to both androgens and glucocorticoids *in vitro* (6), it may be necessary in future studies to adrenalectomize the mice, thereby reducing endogenous glucocorticoid levels, to study the effect of exogenously administered dexamethasone on the −426/28 PB fragment in noncastrated males.

It had been previously observed that in the 12-day castrated rat, despite a 4-fold decrease in dorsolateral prostate weight, PB mRNA returned to precastration levels, possibly through a mechanism mediated in part by adrenal steroids (5, 11). Although a concomitant rebound of PB protein was not characterized in the transgenic mice, PB-directed expression of CAT activity appeared to increase only slightly in the lateral and ventral prostate 10 days postcastration. No further increase was observed in the mice even 22 days after castration. Further studies will be necessary to determine how resistance of dorsolateral prostatic epithelium to androgen ablation might result in the reinduction of PB-directed transcription or the stabilization of mRNAs carrying PB sequence.

As the endogenous PB gene is expressed only at low levels in the prepubertal ventral prostate, and this expression is down-regulated in the postpubetal rat, it was interesting to observe the high level expression of the PB-CAT transgene in the ventral prostate of mature males. Previous reports have demonstrated that the *cis*-acting elements within the −596/21 fragment of the rat fatty acid-binding protein (l-FABP) gene were sufficient to direct efficient cell-specific expression of human (h) GH reporter fusion genes to the enterocytes and hepatocytes in transgenic mice (19), although inappropriate hGH expression was observed in the intestinal crypts, colon, and renal proximal tubular epithelial cells. Although the addition of more 5′-flanking sequence (the −4000/−597 fragment) was sufficient to suppress the expression of hGH in the renal cells, the anomalous expression in fetal liver was not altered (19). It will be interesting to determine whether more of the rat PB 5′-flanking region, once isolated, will be sufficient to suppress the ventral expression of the PB-CAT construct observed in transgenic mice.

Although the PB-CAT transgene was expressed in a tissue-specific fashion, not all of the lines of mice carrying the transgenes expressed the constructs, suggesting that these minimal tissue-specific promoter elements do not carry dominant *cis*-acting sequences analogous to a locus control region (LCR). First characterized as erythroid-specific DNase-I-hypersensitive sites located within a 15-kb *Bam*HI fragment up-stream of the first gene in the β-globin locus (20), it is believed that LCRs function in a *cis*-dominant fashion to physically establish an open, higher order chromatin structure, which may facilitate the expression of tissue-specific genes during development (21). It is interesting to note that the β-globin LCRs have been demonstrated to be required, but are not sufficient (22), to direct high level, copy number-dependent, site of integration-independent expression in transgenic mice. Other studies have identified and characterized tissue-specific LCRs in the 3′-flanking region of the human keratin 18 gene (23), the 3′-flanking region of the human CD2 gene (24), and within the human α-globin gene cluster (25). It remains to be determined whether prostate-specific LCRs can be identified in the regions flanking the PB gene.

It has been well established that highly variable levels of transgene expression can be attributed to the architecture of the transgene in addition to the influence of the flanking DNA due to the random integration of the transgene into the germ line of transgenic mice (26–28). Using a chicken lysozyme transgene, it has been demonstrated that DNase-I-hypersensitive regions immediately flanking the gene are responsible for defining functional genetic domains (29–31). These flanking regions are genetic elements known to associate with the nuclear scaffold proteins and are referred to as scaffold attachment regions or, more commonly, MARs.

Capable of mediating elevated and position-independent gene activity of lysozyme gene constructs in transfection experiments (7, 8) and in transgenic mice (9), the chicken lysozyme MAR is believed to act as a buffer between the transgene and the flanking chromatin by interacting with the nuclear matrix to establish transcriptionally active functional domains, thereby insulating the transgene from *cis*-acting elements at the site of integration (7, 8, 10, 29, 30). Using a series of transgenes containing whey acidic protein (WAP) genes fused to chicken lysozyme MAR elements, the WAP-MAR transgenes were found to be expressed in all founder lines, and accurate position-independent hormonal and developmental regulation was observed in four of the five lines analyzed (10). This was in marked contrast to previous observations that WAP transgenes without MARs were only expressed in 50% of the transgenic animals, and this expression was promiscuous during pregnancy and lacked appropriate hormonal regulation (10, 32, 33).

To determine whether the chicken lysozyme MAR

could suitably insulate the PB-CAT transgene from position effects and facilitate a more appropriate spatial pattern of expression, PB-CAT was coinjected with the lysozyme MAR to generate three independent lines of bigenic (carrying two transgenes) mice. Consistent with the observations for the WAP-MAR transgenes, CAT activity was detected in the dorsolateral prostate of postpubetal male mice from all three lines, and in contrast to the lines carrying only PB-CAT, the activity between the lowest and highest expressing lines only varied over a 10-fold range. More surprisingly, CAT expression was suppressed in the ventral lobe of the prostate in PB-CAT/MAR bigenic mice. Furthermore, the levels of CAT activity in the lateral and dorsal lobes of line 5638 were about 0.4- and 8-fold higher, respectively, than that in line 4248. These findings very closely approximate the distribution of PB in rat prostatic lobes and show that cointegration of chicken lysozyme MARs may act to suppress ectopic PB-CAT transgene expression.

With this series of experiments we have developed a system for targeting heterologous gene expression specifically to the ductal epithelial cells of the prostate in transgenic mice. Therefore, it should now be possible to design experiments to characterize the molecular and cellular mechanisms governing transformation of the differentiating prostate *in vivo*. Studies are currently underway to study the consequence of targeting oncogenes or mutated forms of tumor suppressor genes to the prostate of transgenic mice, both with and without cointegration of the heterologous MAR, to facilitate the establishment of suitable animal models to identify the biological factors involved in the progression of prostate cancer.

## MATERIALS AND METHODS

### Plasmid Constructions and Generation of Transgenes

The −426/28 PB sequences were fused to the CAT and simian virus-40 cleavage and polyadenylation sequences, as previously described (6). The PB-CAT transgene was separated from pUC119 vector sequences by digestion with *Bam*HI and *Hin*dIII. The chicken lysozyme 5′-MAR was separated from pUC19 vector (29) sequences by digestion with *Bam*HI and *Xba*I. Fragments for microinjection were purified by gel electrophoresis and recovered by adsorption to QIAEX beads (QIAGEN, Inc., Chatsworth, CA) according to manufacturer's recommendations. Transgenic mice were generated, and mouse tail DNA was isolated as described previously (34). PB-CAT and MAR transgene copy numbers were estimated by Southern blotting, as previously described (35). The PCR technique was used to screen for positive transgenic mice, as previously described (36). The primer pairs used in PCR screening were A and B (PB-CAT), and C and D (MAR). The sequences of the synthetic oligonucleotides were as follows (5′→3′): A, CCGG TCGACCGGAAGCTTCCACAAGTGCATTTA; B,GTATATCCAGTGATTTTTTTCTCCA; C, GCGCTGCTGACTTTCTAAACATAAG; and D, GAGCTCACGTTAAGTTTTGATGTGT.

### Transgene Expression Analysis

Tissue extracts were prepared and analyzed as described previously (35). Except where noted, tissues were biopsied at various stages of development from mice under anesthesia, and extracts were prepared by homogenization in 0.25 M Tris-HCl (pH 7.5), followed by heating to 65 C for 10 min. Titrated amounts of protein (up to 100 μg) were used for triplicate determinations of CAT activities, as previously described (6, 35).

### Immunocytochemistry

The genito-urinary tract of 7-week-old male mice was fixed in 10% phosphate-buffered formalin for 2 h at 0–4 C, embedded in paraffin, and sectioned at 4 μm. After dewaxing, immunochemical staining was performed, essentially as previously described (37), using a polyclonal rabbit anti-CAT antibody (5-Prime, 3-Prime, Inc., Paoli, PA) at a dilution of 1:500. The secondary antibody was biotinylated goat antirabbit immunoglobulin G, and it was detected using standard immunoperoxidase procedures, as previously described (38).

### *In Situ* Hybridization

Tissue specimens were embedded in Tissue Tek O.C.T. compound (Miles, Inc., Elkhart, IN) and sectioned as previously described (11). The pM-40 probe (1) containing the PB cDNA was used as template for preparation of the digoxigenin-labeled sense and antisense riboprobes (39). Prehybridization was performed for 1 h at room temperature in a solution containing 50% formamide, 2 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M Na citrate), 10% dextran sulfate, and 250 μg/ml *E. coli* transfer RNA. Hybridization was performed overnight at 42 C in fresh prehybridization solution containing 10 μg/ml of the appropriate riboprobe. Washing was performed at 37 C for 30 min/wash, initially in 2 × SSC and then with decreasing concentrations of SSC to a final wash in 0.5 × SSC. The digoxigenin-labeled probes were detected through an immunochemical reaction with an alkaline phosphatase-conjugated polyclonal antidigoxigenin antibody (Boehringer Mannheim, Indianapolis, IN). Visualization reactions contained 0.3% levamasole to inhibit endogenous alkaline phosphatase activity. Serial sections were stained with hematoxylin and eosin for orientation. All slides were mounted in an aqueous mounting medium containing 20% glycerol in PBS.

### Steroid Regulation of Transgene Expression

Mice were castrated by the scrotal route. Intact males served as controls. Where appropriate, 7-day castrated males were supplemented daily with dexamethasone (Sigma, St. Louis, MO; 3 mg/kg) or testosterone (Sigma; 3 mg/kg) in 0.2 ml 10% ethanol-90% peanut oil as vehicle. Delivery was in the scruff of the neck. Control mice received injections of vehicle only. The mice were killed at the appropriate times, and the tissue distribution of CAT activity was determined, as described above.

### Experimental Animals

In accordance with the NIH Guide for the Care and Use of Laboratory Animals, all experiments were conducted using the highest standard for humane care.

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